NE Volume 18, Number 11, pp 1425–1432
993, J. B. Lippincott Company

Autologous Nucleus Pulposus Induces Neurophysiologic and Histologic Changes in Porcine Cauda Equina Nerve Roots

Kjell Olmarker, MD, PhD, Björn Rydevik, MD, PhD, and Claes Nordborg, MD, PhD*

This article received a 1993 Volvo Award in Basic Sciences

Epidural application of autologous nucleus pulposus in pigs, without mechanical nerve root compression, induced a pronounced reduction in nerve conduction velocity in the cauda equina nerve roots after 1–7 days, compared to epidural application of retroperitoneal fat in control experiments. Histologically, the nerve fiber injury was more pronounced after application of nucleus pulposus than after control tissue application. The results demonstrate that nucleus pulposus may induce nerve tissue injury by mechanisms other than mechanical compression. Such mechanisms may be based on direct biochemical effects of nucleus pulposus components on nerve fiber structure and function and microvascular changes including inflammatory reactions in the nerve roots. [Key words: nerve roots, cauda equina, disc, nucleus pulposus, nerve conduction, neuropathology]

Despite the major socioeconomic and medical significance of low back pain, the knowledge of underlying basic pathophysiologic mechanisms is fairly limited.[30,41] One specific pathoanatomic condition recognized to be related to back pain syndromes, particularly to sciatic pain, is intervertebral disc herniation.[20,29,41] Besides mechanical deformation of the nerve roots, it has also been suggested that various tissue components of the intervertebral disc itself might biochemically affect the nerve roots. Thus, irritation of nerve roots by substances such as glycoproteins,[25,26,32] immunoglobulin G,[38] PLA_2,[43] and hydrogen ions[6,28] has been proposed. It has also been suggested that there might be an autoimmune reaction, based on the fact that the avascular nucleus pulposus is normally secluded from the immune system. Exposure through a herniation might present the nucleus pulposus tissue to the immune system and thus initiate autoimmune reactions.[2,3,10-12,20,31-33] However, none of these theories has been scientifically proven. In the current study, the effects of locally applied autologous nucleus pulposus on the hog cauda equina were analyzed with respect to neurophysiologic and histopathologic changes with the aim of learning if disc herniation might induce nerve injury also through mechanisms other than mechanical deformation.

Material and Methods

A total of 30 hogs, weighing 25–30 kg, were anesthetized by an intramuscular injection of 20 mg/kg/body weight of Ketalar (ketamine 50 mg/ml, Parke-Davis, Morris Plains, New Jersey) and an intravenous injection of 4 mg/kg/body weight of Hypnodil® (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0.1 mg/kg/body weight of Stresnil® (azaperon 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). Anesthesia was maintained by additional intravenous injections of 2 mg/kg/body weight of Hypnodil® and 0.05 mg/kg/body weight of Stresnil®. The mean arterial blood pressure was continuously monitored by a catheter in the thoracic aorta connected to a Gould Statham P23 pressure transducer (Gould Statham Instruments Co, Hato Rey, Puerto Rico) and a Grass 7B polygraph recorder (Grass Instrument Co., Quincy, MA). One intramuscular injection of 10 mg/kg of Novocillin® (Novo Nordiska A/S) was given just before and after surgery. The hogs also received an intravenous injection of 0.1 mg/kg of Stesolid Novum® (Dumex, Helsingborg) after surgery.

The hogs were placed on their side and the lumbar spine was exposed by a retroperitoneal approach (Figure 1). The L3–4 intervertebral disc was incised laterally and the nucleus pulposus was harvested. The wound was sutured and the hogs were placed in a prone position. A bilateral laminectomy of the second coccygeal vertebra, not interfering with the facet joints, was performed through a midline incision. The cauda equina was exposed and the nucleus pulposus was gently placed epidurally in close contact with the spinal nerve roots. The lamina were removed, so

From the Department of Orthopaedics and *Department of Pathology, University of Gothenburg, Sahlgren Hospital, Gothenburg, Sweden.
Supported by grants from the Swedish Medical Research Council (8685, 9758), the Greta och Einar Asker Research Foundation, the Carin Trygger Memorial Foundation, the Inga Britt and Arne Lundberg Research Foundation, the Félix Neubergh Foundation and the Tore Nilsson Research Foundation.
Accepted for publication March 29, 1993.

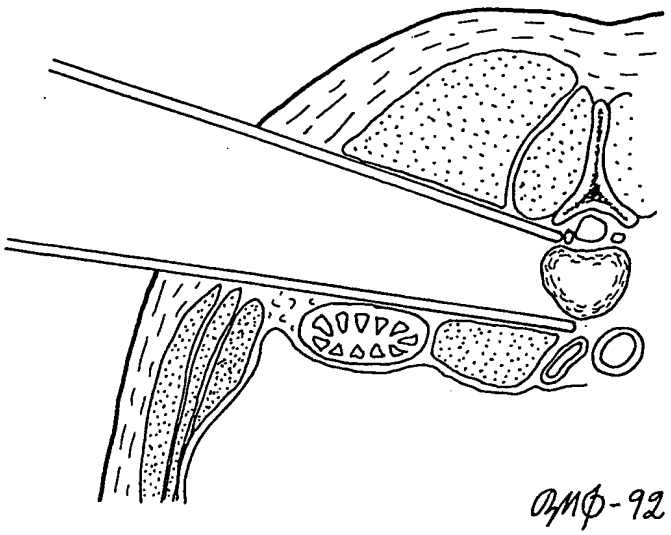

Figure 1. Schematic drawing of retroperitoneal approach for harvesting nucleus pulposus from the L3–4 intervertebral disc.

enough room was provided for the nucleus pulposus to avoid mechanical compression of the cauda equina. In control animals the same harvesting procedure was performed. However, the nucleus pulposus material was not used. Instead, the same volume of retroperitoneal fat was harvested and placed on the cauda equina instead of the nucleus pulposus. Both nucleus pulposus and retroperitoneal fat were in a semiliquid state. There were five animals in each of the six different subseries. The experimental protocol was approved by the animal ethics research committee, University of Gothenburg.

Analyses were performed after 1, 3, and 7 days. The hogs were then reanesthetized by an intramuscular injection of 20 mg/kg/body weight of Ketalar and an intravenous injection of 35 mg/kg/body weight of Pentothal (thiopental sodium, Abbott Laboratories, Chicago, IL). The hogs were ventilated on a respirator. Anesthesia was maintained by an intravenous bolus injection of 100 mg/kg/body weight of Chloralose ((a)-D(+)-gluco-chloralose, Merck, Darmstadt, Germany) and by a continuous intravenous infusion of 30 mg/kg/hour of Chloralose. A laminectomy from the fifth sacral to the fourth coccygeal vertebra was performed. The preparation was covered with Spongostane® (Ferrosan, Denmark) to maintain temperature and moisture. Local tissue temperature was continuously monitored and maintained at 37.5–38.0°C by means of a heating lamp.

Neurophysiologic Assessment. The cauda equina was stimulated by two E2 subdermal platinum needle electrodes (Grass) that were connected to a Grass SD9 stimulator (Grass) and gently placed intermittently on the cauda equina both cranial and 30 mm caudal to the exposed area. An electromyogram (EMG) was registered by two subdermal platinum needle electrodes, which were placed into the paraspinal muscles in the tail approximately 10 mm apart (Figure 2). This procedure is reproducible and represents a functional measurement of the motor nerve fibers of the cauda equina nerve roots.[34,35,37,42] The EMG was visualized using a MacIntosh IIci computer provided with Superscope software and MacADIOS II A/D converter (GW Instruments, Sommerville, Mass.) together with a Grass P18 preamplifier. The separation distance between the first peaks of the EMG from the two recordings was determined and the separation distance between the two stimulation sites on the cauda equina was measured with calipers. The nerve conduction velocity between the two stimulation sites could thus be calculated from these two measurements.

To ensure that only impulses from exposed nerve fibers were registered, all nerves that left the spinal canal between the cranial stimulating electrodes and the exposure site were cut. A confirmation of the outcome of this procedure was obtained after the experiment by studying the EMG after cutting the cauda equina at the compression site, which always resulted in a flat EMG curve.

The difference between nucleus pulposus exposure and control at corresponding times was analyzed using Student *t* test for unpaired samples.

Histologic Assessment. The cauda equina was tied to a wooden stick to avoid shrinkage artifacts and was fixed by immersion in Karnovsky's mixture of formaldehyde and glutaraldehyde.[18] Specimens for microscopy were obtained from the exposed segments of the cauda equina. The specimens were dehydrated and embedded in Epon 812. Semithin sections (1-μm thick) were prepared and stained according to Richardson.[39] All sections were coded and analyzed by means of light microscopy by one neuropathologist, who thus was unaware of the test protocol for the different specimens. The data were organized in tables after code breakage.

■ Results

No hogs showed evidence of pain or neurophysiologic deficit after the first day of surgery as assessed by the animal keepers. No postoperative wound infections were noted.

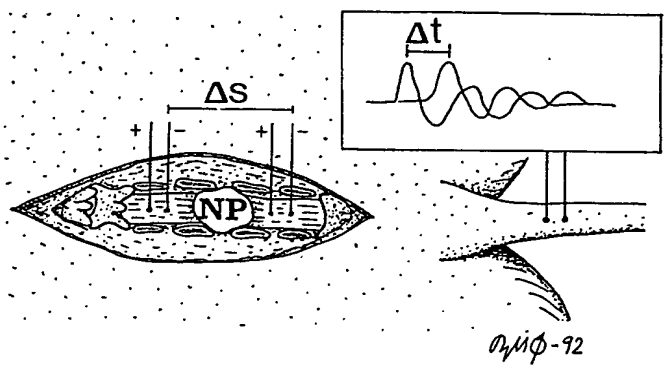

Figure 2. Procedure for determination of nerve root conduction velocity. The cauda equina is stimulated cranial and caudal to the segment exposed to nucleus pulposus or control. The difference in distance (Δs) between these two stimulation sites is divided by the time difference (Δt) between the first peaks of the two recordings.

Table 1. Nerve Root Conduction Velocity (m/s ± SD)

Series	1 Day	3 Days	7 Days
Control (fat)	84 ± 2	83 ± 4	76 ± 11
Nucleus pulposus	63 ± 9	45 ± 16	45 ± 19

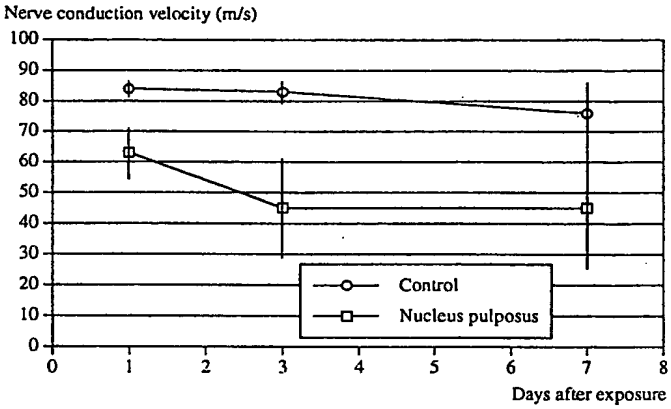

Figure 3. Nerve conduction velocity for nerve roots exposed to retroperitoneal fat (control) and nucleus pulposus. Error bars indicate standard deviation.

Neurophysiologic Assessment

Nerve conduction velocities for the different series are presented in Table 1 and Figure 3. The conduction velocity was significantly lower in the nucleus pulposus exposed nerve roots than in the fat exposed (control) nerve roots after corresponding exposure durations ($P < 0.01$ at days 1 and 3, $P < 0.05$ at day 7).

Histologic Assessment

The tissue samples were examined by light microscopy according to 13 different morphologic parameters (Tables 2–5). Hyperemia was seen in the epidural tissue in all animals but one and epidural bleeding was found in all specimens; there were no differences between the categories. Neither was there any obvious difference in epidural leukocyte or monocyte infiltration. The most marked fibroblast proliferation was found after 7 days in both groups. The mast cells were mainly found in the dura mater covering the filum, and were most common after 3 days in animals exposed to nucleus pulposus and after 7 days when fatty tissue had been applied (Tables 2–5).

The endoneurial hyperemia was, on average, somewhat more pronounced after the application of nucleus pulposus. Endoneurial bleedings were uncommon and rather discrete in both groups. The assessment of nerve fiber damage was restricted to myelinated fibers. Although a sometimes marked nerve fiber degeneration was noticed in nine animals exposed to nucleus pulposus, only one animal showed slight damage after application of fatty tissue. Signs of ongoing nerve fiber degeneration were axonal swelling, increased axoplasmic density, marked attenuation or splitting of the myelin sheaths, and the appearance of Schwann cells containing myelin fragments (Figure 4, A and B). Moreover, there was a sometimes very marked swelling and attenuation of the Schwann cell cytoplasm outside the myelin sheath in fibers that otherwise appeared normal (Figure 5A). This phenomenon was slightly more common after the application of nucleus pulposus. Vacuoles within the axons or seemingly between the axons and the myelin sheaths (Figure 5, B and C) were more common in the group exposed to fatty tissue (Tables 2–5).

■ Discussion

The results of the current study demonstrate a significant nerve conduction velocity reduction and nerve

Table 2. Epidural Histologic Changes: Nucleus Pulposus

	Hyperemia	Bleeding	Leukocytes	Monocytes	Fibroblasts	Mastcells	Nerve Conduction Velocity (m/s)
7 days	(+)	+	0	+	+++	0	60
	(+)	+	0	+	+++	0	14
	+	+	+	+	0	(+)	41
	+	+	+	+	+	(+)	58
	+	+	+	0	0	+	54
3 days	+	+	0	+	+	+	47
	+	+	+	+	+	+	67
	+	+	+	+	+	+	25
	-	(+)	+	+	+	+	48
	+	+	+	+	0	+	36
1 day	+	+	+	+	0	0	69
	+	+	+	+	+	+	53
	+	+	+	+	+	0	70
	+	+	+	+	0	0	55
	+	+	+	+	+	0	70

Grading system: 0 = no changes, (+) = diminutive changes, + = mild changes, ++ = moderate changes, +++ = severe changes.

Table 3. Epidural Histologic Changes: Retroperitoneal Fat (Control)

	Hyperemia	Bleeding	Leukocytes	Monocytes	Fibroblasts	Mastcells	Nerve Conduction Velocity (m/s)
7 days	+	+	+	+	+	0	87
	+	+	+	+	0	0	82
	+	(+)	+	0	0	+	82
	+	+	+	+	+++	+	59
	+	+	+	+++	+++	+++	70
3 days	+	+	+	+	+	0	86
	+	+	+	+	0	0	85
	+	+	0	+	+	(+)	76
	+	+	0	+	0	(+)	82
	NV	+	0	+	0	0	84
1 day	+	+	+	+	0	0	86
	+	+	0	+	+	(+)	82
	+	+	+	+	+	(+)	84
	+++	+	+	+	0	(+)	86
	+	+	+	+	0	0	81

Grading system: 0 = no changes, (+) = diminutive changes, + = mild changes, ++ = moderate changes, +++ = severe changes.

fiber degeneration after epidural application of autologous nucleus pulposus onto the sacrococcygeal cauda equina, without mechanical compression, as compared to control. This effect was present after 1 day of exposure and persisted throughout the observation time of 7 days. To our knowledge such an effect of nucleus pulposus on nerve root structure and function has not been demonstrated before. Various mechanisms may be involved in the pathophysiology of such phenomena, for example direct effects of nucleus pulposus components on nerve fibers or vascular and inflammatory reactions.

Many authors have reported signs of nerve root inflammation in certain cases of disc herniation. This has been thought to be the result of irritation of nerve tissue and meninges induced by the herniated intervertebral disc tissue.[1,4,9,10,13,15,19,21,22,25,26,33,44,45] Some specific substances in the nucleus pulposus have been suggested to be responsible for such chemical irritation of the nerve roots. Diamant et al and Nachemson et al found that a low pH and increased levels of lactate in the nucleus pulposus correlated with the degree of connective tissue reactions around the nerve roots in patients operated for lumbar rhizopathies.[6,28] Marshall et al have suggested that glycoproteins from the disc may have nerve irritating properties.[25,26] Incidentally, glycoproteins have also been found in nerve roots exposed to herniated disc material.[32] Recently, Pennington and collaborators[38] discovered immunoglobulin G molecules in dog discs.[38] The presence of immunoglobulin G was suggested to make it possible for nucleus pulposus to in-

Table 4. Endoneural Histologic Changes: Nucleus Pulposus

	Hyperemia	Bleeding	Fiber Injury	Schwann Cell Edema	Axonal Vacuolization	Nerve Conduction Velocity (m/s)
7 days	+	0	+	+	(+)	60
	+	+	+++	0	+	14
	0	0	(+)	+	+	41
	+	0	0	+	+	58
	+	0	(+)	0	(+)	54
3 days	0	0	+	+	+	47
	+	0	+	+	(+)	67
	+	+	+	+	(+)	25
	0	0	0	+	(+)	48
	+	0	0	+	+	36
1 day	0	0	0	+	(+)	69
	0	0	0	+	(+)	53
	+++	0	+	+	(+)	70
	+	+	+	0	+	55
	-	0	0	+	(+)	70

Grading system: Hyperemia and bleeding: 0 = no changes, + = mild changes, ++ = moderate changes, +++ = severe changes.Fiber injury. Schwann cell edema and axonal vacuolization: 0 = no changes, (+) = single, very few of myelinated fibers, + = <10% of myelinated fibers, ++ = 10<25% of myelinated fibers, +++ = 25<50% of myelinated fibers, ++++ = 50<75% of myelinated fibers, +++++ = 75–100% of myelinated fibers.

Table 5. Endoneural Histologic Changes: Retroperitoneal Fat (Control)

	Hyperemia	Bleeding	Fiber Injury	Schwann Cell Edema	Axonal Vacuolization	Nerve Conduction Velocity (m/s)
7 days	0	0	0	0	+	87
	0	0	0	0	(+)	82
	0	0	0	0	+	82
	0	0	+	+	+	59
	+	0	0	+	(+)	70
3 days	+	0	0	+	++++	86
	+	0	0	+	+	85
	+	0	0	0	+	76
	0	+	0	+	+++	82
	0	0	0	+	+++	84
1 day	+	0	0	+	+++	86
	0	0	0	+	+	82
	+	0	0	0	(+)	84
	0	+	0	(+)	+	86
	+	0	0	0	(+)	81

Grading system: Hyperemia and bleeding: 0 = no changes, + = mild changes, ++ = moderate changes, +++ = severe changes.Fiber injury, Schwann cell edema and Axonal vacuolization: 0 = no changes, (+) = single, very few of myelinated fibers, + = <10% of myelinated fibers, ++ = 10<25% of myelinated fibers, +++ = 25<50% of myelinated fibers, ++++ = 50<75% of myelinated fibers, +++++ = 75–100% of myelinated fibers.

duce inflammatory changes in tissues such as nerve roots. Human phospholipase A2 is an enzyme that regulates the arachidonic acid cascade and plays an essential role in the inflammatory process. It has been found to be present in human discs removed for radiculopathy, which further suggests that disc tissue might induce inflammatory reactions in or around nerve roots at disc herniation.[43] The latest potentially irritating substance that has been found in disc tissue is stromelysin.[23] This enzyme is considered to be responsible for generating molecular heterogeneity, thus relating to degenerative changes in the disc.

Because the nucleus pulposus is normally not in contact with the systemic circulation after its embryologic formation, it might theoretically also induce an autoimmune reaction if it is presented to the immune system later in life at a herniation.[14,20,31] Bobechko and Hirsch[3] found a response of lymphocytes in primary lymph nodes after injection of autologous nucleus pulposus in rabbits ear. This reaction was maximal 4

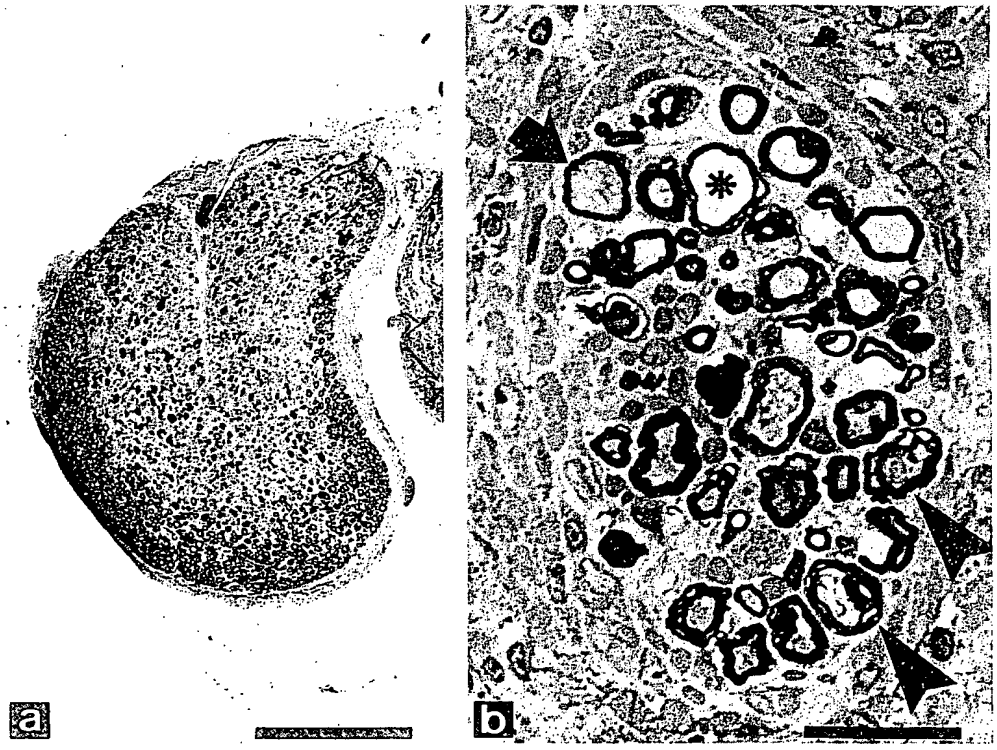

Figure 4. **A**, Marked nerve fiber degeneration 7 days after epidural application of nucleus pulposus. The loss of nerve fibers is more marked in the central part of the root, (Richardson, Bar 260 μm). **B**, Nerve fiber degeneration in small nerve root 3 days after application of nucleus pulposus. Nerve fibers with increased axonal density and loose (arrow-heads) or thin (arrow) myelin sheaths are seen as well as myelinated fibers with markedly attenuated axoplasm (star), (Richardson, Bar 26 μm).

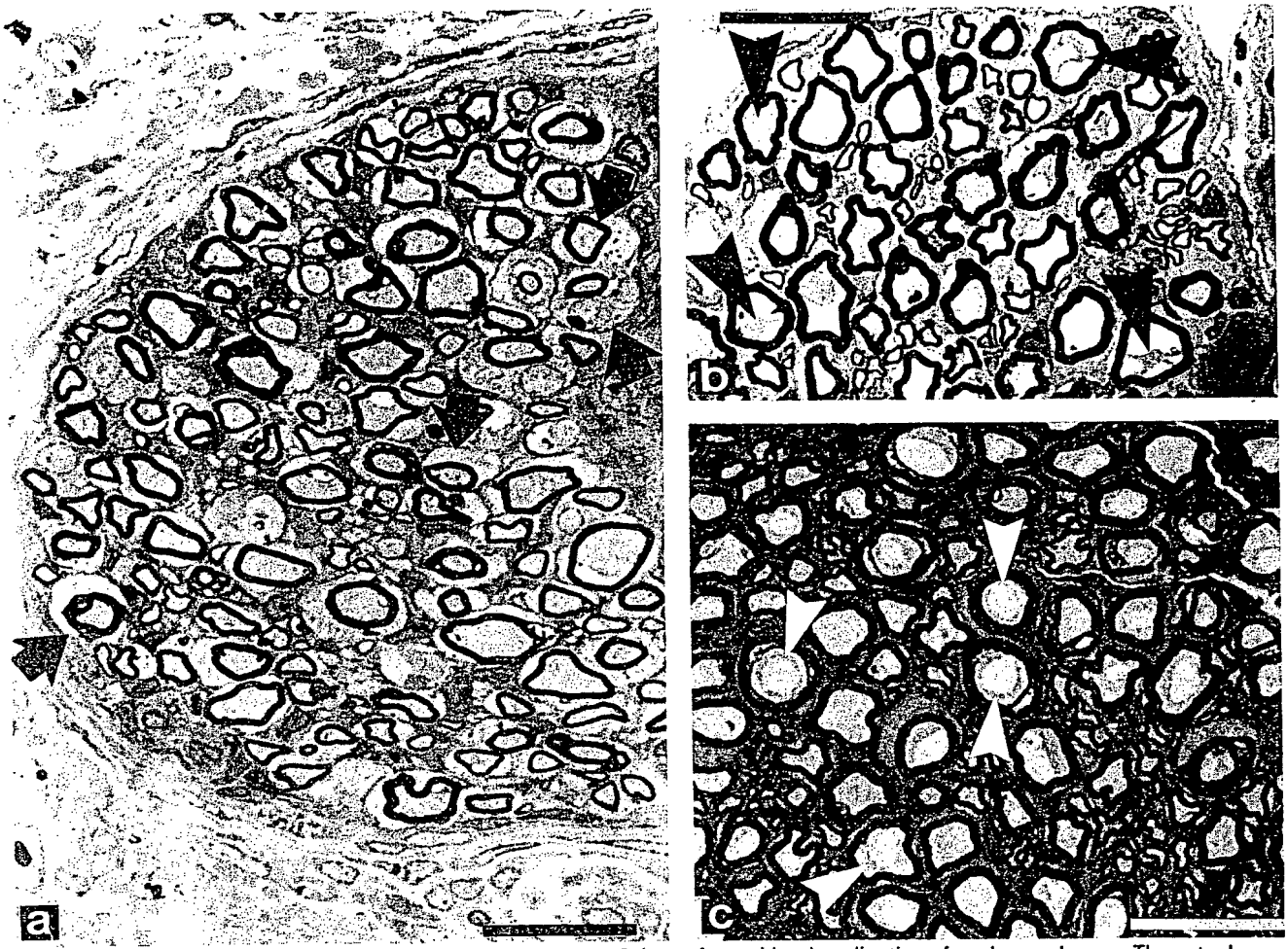

Figure 5. **A**, Swelling of the Schwann cell cytoplasm in a nerve root 3 days after epidural application of nucleus pulposus. The cytoplasm outside the myelin sheath is markedly expanded and pale (arrows) in a majority of the fibers, (Richardson, Bar 26 mm). **B** and **C**, Nerve root 3 days after epidural application of retroperitoneal fat. Some axons appear partly collapsed and loosened from the inner surface of the myelin sheath (black arrow-heads). Large vacuoles (white arrow-heads) are seen in other myelinated axons, (Richardson, Bar 26 mm).

days after injection and persisted for at least 3 weeks. Human nucleus pulposus has also been found to be able to raise "antisera" when injected into rabbits.[36] Lundskog and Brånemark[24] inserted autologous nucleus pulposus tissue subperichondrally in rabbit ears. No round cell infiltration or lymphocytic reactions were observed, but the authors concluded that the vascular reaction pattern might indicate an autoimmune reaction. Some studies have demonstrated a positive leukocyte migration inhibition test in patients with sequestrated nucleus material, which might indicate an immunologic reaction.[10,11,8] Naylor and collaborators[33] could not demonstrate any antibodies in the nucleus pulposus but observed, as well as Bisla and collaborators,[2] an increase of immunoglobulin M in protein immunoelectrophoresis. De Silva and collaborators,[5] however, could not demonstrate any lymphocyte transformation or any increase of immunoglobulin M, immunoglobulin G, immunoglobulin A, or C-reactive protein in serum of patients with surgically proven herniated intervertebral discs. The authors concluded that an autoimmune reaction was not likely.

In the current investigation autologous nucleus pulposus applied epidurally to the sacrococcygeal cauda equina in hogs induced pronounced effects on the structure and function of the spinal roots. There was thus a marked reduction in nerve conduction velocity as well as nerve fiber degeneration in spinal nerve roots exposed to nucleus pulposus. However, even in the series with the most pronounced damage, breakdown of axons and/or myelin sheaths was only seen in a minority of the myelinated nerve fibers, which indicates that the cause of the conduction disturbance may be complex. The distribution of nerve fiber damage was central in one nerve root after application on nucleus pulposus (Figure 4A). Central fascicular lesions are seen when the en-

doneurial circulation is compromised by vasculitis and may thus indicate an element of ischemia.[7] The epineurial and endoneurial bleedings as well as the hyperemia likewise indicate a pronounced disturbance of blood flow, which most likely could influence the function of nerve fibers. Also, other findings, such as the Schwann cell edema, might be of importance; electron microscopic analysis will be needed to investigate if there is also an intra-myelin edema. The axonal vacuoles are probably of minor physiologic importance, because they were most prominent in the control series, with its relatively fast conduction velocities. Autologous nucleus pulposus application to rabbit tibial nerves did not cause any structural nerve fiber damage or functional impairment,[40] which may be due to the presence of perineurial sheaths with protective diffusion barrier properties around the peripheral nerve fascicles.[41] Spinal nerve roots do not have perineurial sheaths;[35,41] the roots exhibit less efficient surface diffusion barriers, which thus may allow passage of various substances, for example from nucleus pulposus to the nerve tissue.

The question of whether the noticed epidural inflammatory reaction could impair nerve conduction by changing the endoneurial milieu needs further elucidation. Jaffray and O'Brien[16] found inflammatory changes in the anterior annulus fibrosus in patients undergoing surgery for pain relief due to resorption of an intervertebral disc. McCarron and collaborators[27] performed a histologic study in dogs in which autologous nucleus pulposus from the amputated tail discs was injected epidurally over a period of 7 days. Specimens were analyzed at times ranging between 4 and 21 days. The nucleus pulposus induced an epidural inflammatory reaction, which was absent in dogs injected with saline for control. According to the results of the present study there was an epidural inflammatory reaction after application of both nucleus pulposus and fat. One might suspect that the observed inflammatory effect is not specific for nucleus pulposus and that saline for that reason was not a relevant control. The inflammatory reaction may instead be a common reaction to any tissue that is not normally present in the spinal canal. Conversely, an inflammatory reaction may be of pathologic significance, regardless of its cause. Disturbances in the fibrinolytic system have been implicated in chronic nerve root injury and sciatica.[17] One may also speculate that the observed effects of nucleus pulposus on nerve root conduction properties may in part be related to the negative charges of disc proteoglycans, affecting the nerve fiber membrane potentials and thereby the impulse conduction properties.

In conclusion, the results of this study demonstrate that epidural application of autologous nucleus pulposus without mechanical compression may induce pronounced changes in nerve root structure and function. The pathophysiology of such effects of nucleus pulposus on nerve tissue is likely to be complex, comprising factors such as directly irritating substances, autoimmune reactions, microvascular changes, and inflammatory phenomena.

References

1. Akeson WH, Woo SL, Taylor TKF, Gosch P, Bushell GR: Biomechanics and biochemistry of the intervertebral disk. Clin Orthop 129:133–140, 1977
2. Bisla RS, Marchisello PJ, Lockshin MD, et al: Autoimmunological basis of disk degeneration. Clin Orthop 123:149–154, 1976
3. Bobechko WP, Hirsch C: Auto-immune response to nucleus pulposus in the rabbit. J Bone Joint Surg 47B:574–580, 1965
4. Braun IF, Lin JR, Benjamin MU, Kriecheff II: Computed tomography of the asymptomatic postsurgical lumbar spine: Analysis of the physiologic scar. Am J Roentgenol 142:149–152, 1984
5. De Silva M, Hazleman BL, Ward M, Park JR, Dickson I: Auto-immunity and the prolapsed intervertebral disc syndrome. Rheumatol 1:35–38, 1981
6. Diamant B, Karlsson J, Nachemson A: Correlation between lactate levels and pH in discs of patients with lumbar rhizopathies. Experienta 24:1195–1196, 1968
7. Dyck PJ, Karnes J, Lais A, Lofgren EP, Stevens JC: Pathologic alterations of the peripheral nervous system of humans. Peripheral Neuropathy. Volume 1. Second edition. Edited by PJ Dyck, PK Thomas, EH Lambert, R Bunge. Philadelphia, WB Saunders, 1984, pp 828–830
8. Elves MW, Bucknill T, Sullivan MF: In vitro inhibition of leucocyte migration in patients with intervertebral disc lesions. Orthop Clin North Am 6:59–65, 1975
9. Fernström U: Disk rupture with surgical observations. Acta Chir Scand Suppl 258; 14–25, 1960
10. Gertzbein SD, Tile M, Gross A, Falk R: Autoimmunity in degenerative disc disease of the lumbar spine. Orthop Clin N Am 6:1:67–73, 1975
11. Gertzbein SD: Degenerative disk disease of the lumbar spine. Immunological implications. Clin Orthop 129:69–71, 1977
12. Gertzbein SD, Tait JH, Devlin SR: The stimulation of lymphocytes by nucleus pulposus in patients with degenerative disk disease of the lumbar spine. Clin Orthop 123:149–154, 1977
13. Halperin N, Agasi M, Hendel D: Painless root compression following disc extrusion. Arch Orthop Trauma Surg 101:63–66, 1982
14. Hirsch C, Schajowicz: Studies on structural changes in the lumbar annulus fibrosus. Acta Orthop Scand 22:184–231, 1952
15. Holmes JM, Sworn BR: Lumbosacral root pain. Br Med J 1:946, 1946
16. Jaffray D, O'Brien JP: Isolated intervertebral disc resorption. A source of mechanical and inflammatory back pain? Spine 11:397–401, 1986
17. Jayson MIV: Vascular damage fibrosis and chronic inflammation in low back pain and sciatica. Chap 6. The Lumbar Spine and Back Pain. Edited by MIV Jayson. London, Churchill Livingstone, 1992, pp 101–109
18. Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27:137A, 1965.
19. Kelly M: Is pain due to pressure of nerves? Neurology 6:32–36, 1956

20. LaRocca H: New horizons in research on disc disease. Orthop Clin North Am 2:521, 1971
21. Lewin P: Backache and sciatic neuritis. Philadelphia, Lea and Febinger, 1943
22. Lipson SJ, Muir H: Proteoglycans in experimental intervertebral disc degeneration. Spine 6:194–210, 1981
23. Liu J, Roughley PJ, Mort JS: Identification of human intervertebral disc stromelysin and its involvement in matrix degeneration. J Orthop Res 9:568–575, 1991
24. Lundskog J, Brånemark PI: Microvascular proliferation produced by autologous grafts of nucleus pulposus. Adv Microcirc 3:115–124, 1970
25. Marshall LL, Trethewie ER: Chemical irritation of nerve-root in disc prolapse. Lancet ii:320, 1973
26. Marshall LL, Trethewie ER, Curtain CC: Chemical radiculitis. Clin Orthop 129:61–67, 1977
27. McCarron RF, Wimpee MW, Hudkins P, Laros GS: The inflammatory effect of nucleus pulposus. A possible element in the pathogenesis of low-back pain. Spine 12:8:760–764, 1987
28. Nachemson A: Intradiscal measurements of pH in patients with lumbar rhizopathies. Acta Orthop Scand 40:23–42, 1969
29. Nachemson AL: The lumbar spine, an orthopaedic challenge. Spine 1:59–71, 1976
30. Nachemson AL: Spinal disorders. Overall impact on society and the need for orthopedic resources. Acta Orthop Scand 62(Suppl 241):17–22, 1991
31. Naylor A: The biophysical and biochemical aspects of intervertebral disc herniation and degeneration. Ann R Coll Surg Engl 31:91–114, 1962
32. Naylor A: Biochemical changes in human intervertebral disk degeneration and prolapse. Orthop Clin North Am 2:2:343, 1971
33. Naylor A, Happey F, Turner RL, et al: Enzymatic and immunological activity in the intervertebral disc. Orthop Clin North Am 6:51–58, 1975
34. Olmarker K, Holm S, Rydevik B. Importance of rate of onset of compression for the degree of impairment of impulse propagation in experimental compression injury of the pig cauda equina. Spine 15:416–419, 1990
35. Olmarker K: Spinal nerve root compression. Nutrition and function of the porcine cauda equina compressed in vivo. Acta Orthop Scand Suppl 242:1–23, 1991
36. Pankovitch AM, Korngold L: A comparison of the antigen properties of nucleus pulposus and cartilage protein polysaccharide complexes. J Immunol 9:431–437, 1967
37. Pedowitz RA, Garfin SR, Hargens AR, et al: 1990. Effects of magnitude and duration of compression on spinal nerve root conduction. Spine 17:194–199, 1992
38. Pennington JB, McCarron RF, Laros GS: Identification of IgG in the canine intervertebral disc. Spine 13:909–912, 1988
39. Richardsson KC, Jarett L, Finke EH: Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol 35:313–323, 1960
40. Rydevik B, Brown MD, Ehira T, Nordborg C, Lundborg G.: Effects of graded compression and nucleus pulposus on nerve tissue—an experimental study in rabbits. Proceedings of the Swedish Orthopaedic Association, Göteborg, Sweden. Acta Orthop Scand 54:670–71, 1983
41. Rydevik B, Brown MD, Lundborg G: Pathoanatomy and pathophysiology of nerve root compression. Spine 9:7–15, 1984
42. Rydevik BL, Pedowitz RA, Hargens AR, et al: Effects of acute graded compression on spinal nerve root function and structure: An experimental study on the pig cauda equina. Spine 16:487–493, 1991
43. Saal JS, Franson RC, Dobrow R, et al: High levels of inflammatory phospholipase A2 activity in lumbar disc herniations. Spine 15:674–678, 1990
44. Smyth MJ, Wright V: Sciatica and the intervertebral disc. An experimental study. J Bone Joint Surg 40A:1401–1418, 1958
45. Walk L: Diagnostic lumbar disc puncture. Clinical review and analysis of sixty-seven cases. AMA Arch Surg 66:232, 1953

Address reprint requests to

Kjell Olmarker MD, PhD
Department of Orthopaedics
Sahlgren Hospital
Gothenburg University
S-413 45 Gothenburg, Sweden